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FINAL TECHNICAL REPORT

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Metabolic Changes and Hemodynamic Dysfunction

Following Hypothermic Shock

Grant Number: N00014-89-J-3124

Harvey I Miller, Ph.D. - Principal Investigator

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June 30, 1993

Sudden chilling of the entire body by immersion in a very cold water environment, overwhelms the temperature regulatory system and the core temperature starts falling rapidly. When the core temperature falls below 35°C, the individual is considered to be hypothermic. There are two classes of hypothermia, iatrogenic and accidental. The iatrogenic type refers to lowering the body temperature to aid surgical or chemical therapy in cardiac, neurologic or neoplastic diseases. Accidental hypothermia is a nonpurposeful lowering of the body temperature due to untoward circumstances which place the individual in a situation in which the ambient temperature is so low that the normal temperature regulatory mechanisms of the body cannot work. Accidental hypothermia can be further divided into 3 levels: mild (temperature between 35°C and 32°C) moderate (temperature between 32°C and 28°C) and severe (temperature below 28°C). Because of the nature of the military's mission, the possibility of becoming severely hypothermic is common. The usual treatment is to warm the body by external heat (electric blankets, covers, warm water, etc.), peritoneal lavage and cardiovascular bypass. There is apparently no difference between the various rewarm procedures as to recovery and survival. However, many individuals survive the

initial insult only to succumb 2 or 3 days later to cardiogenic shock. This led us to the hypothesis that: "Acute severe accidental hypothermia produces long term perturbations which may produce permanent injury or death."

ANIMAL MODEL

The first year was spent in designing and modifying an animal model. We had used the guinea pig in several shock models, because its cardiovascular system was very similar to human function. Our model for these studies was a guinea pig with an arterial and venous catheter in the carotid artery and jugular vein. A micro thermistor was placed in the other carotid artery and maneuvered into the arch of the aorta. The guinea pig can tolerate having both of its carotid arteries tied off because more than 80% of the blood flow to the brain is by the vertebral arteries and they have a complete Circle of Willis.

The catheters were placed under Ketamine/Rompun - anesthesia using aseptic conditions. Several days after surgery, when the animals body temperature was normal (38°C) and they were gaining weight, we used them in our experiments.

The actual experiments had been modified since the inception of these studies. After making vital measurements (blood pressure, cardiac output using thermal dilution, heart rate and a blood sample (about 1.5 ml), the guinea pig was anesthetized with Ethrane. In more recent experiments the anesthetic had been changed to an anesthetic called Brevital®, a fast acting barbiturate. With the animal in deep but very temporary narcosis, it was placed in an ice water bath up to its neck. Temperature changes were followed from the thermistor in the arch of the aorta. When the core body temperature fell to 25°C, the animal was removed from the bath, dried and wrapped in a heating pad. The body temperature continued to fall for the first couple of minutes. If the core temperature fell below 20.5, the heart stopped and we could not start it again. In most of the experiments, the body temperature (BT) of the animals used did not go that low. During the cooling and rewarm several measurements and samples were taken. They were also taken at various time points up to 48 hours.

Some animals were anesthetized with sodium pentobarbital at various time intervals and the hearts excised and hung on an

isolated, perfused working heart apparatus. Various ventricular functions tests were performed.

FIGURE 1

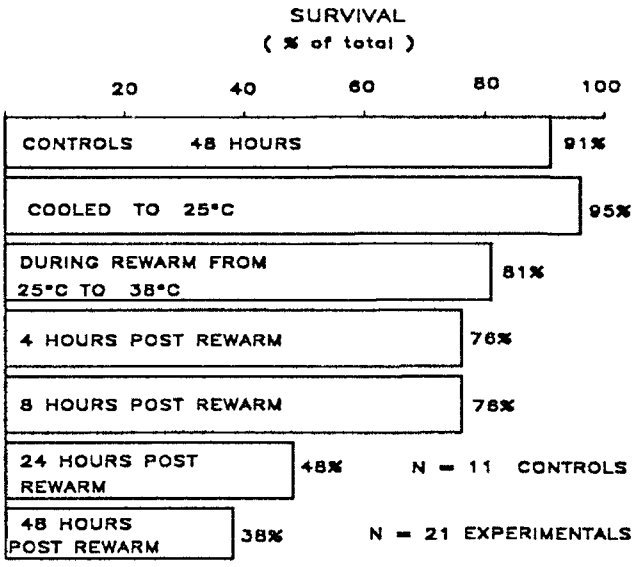


Figure 1 shows the % survivors at various time intervals. One of 11 controls (anesthetized and immersed in 38°C water) died during a 48 hour period after the end of the rewarm period probably due to thrombi. One out of 21 experimental animals died while they were being cooled to 25°C. Eighty one percent of those left survived the rewarm period, while 76% made it to 8 hours. About 50% of the survivors died within 24 hours and 38% of those that made it to 24 hours survived to 48 hours. Those that survived 48 hours appeared to be normal and the few we observed for longer periods, up to 2 weeks, appeared to be normal.

FIGURE 2

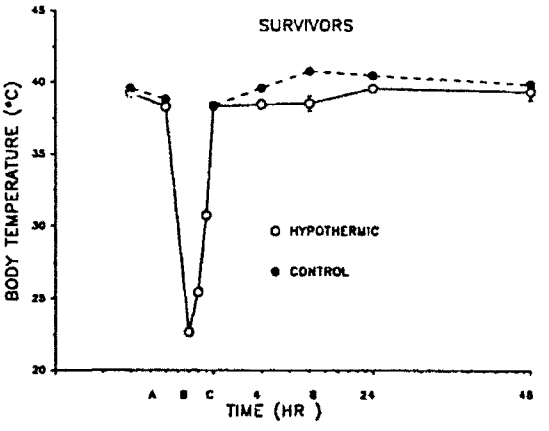


Figure 2 shows the changes in core temperature for both control and hypothermic animals. The thermistor was in the arch of the aorta. There were little changes in control temperature (closed circles). The guinea pigs in the ice water bath had a rapid loss of temperature. They were removed from the bath when the body temperature dropped to 25°C but their BT continued to fall. The lowest mean BT was 22.5°C. BT rose rapidly to 38.6°C while each animal was wrapped in a heating pad, and remained stable over the next 48 hours.

HEMODYNAMIC MEASUREMENTS

The heart rate followed the same pattern as the BT and fell rapidly in the experimental animals while the control animals body temperature fell slightly after anesthesia (Figure 3) but returned to normal at recovery. Cardiac output (Figure 4) also fell during cooling and returned to normal values during rewarming period. It fell again at 4 hours and rose again at 8. By 24 hours it was stable. Blood pressure fell following application of anesthesia in both controls and hypothermic(Fig 5). This change appeared to be due to mostly an affect of the anesthesia.

FIGURE 3

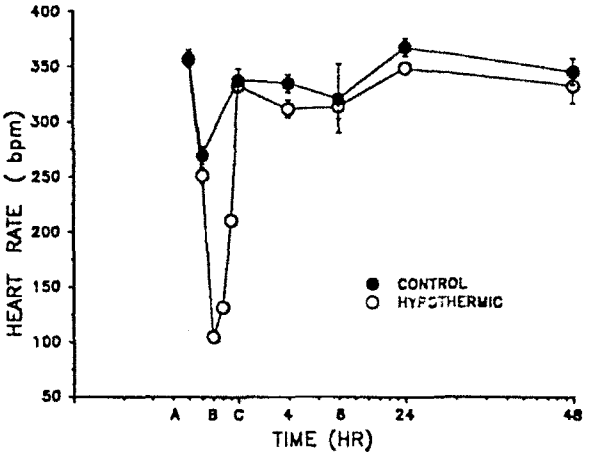


FIGURE 4

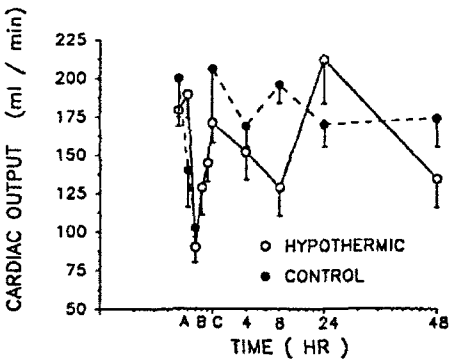
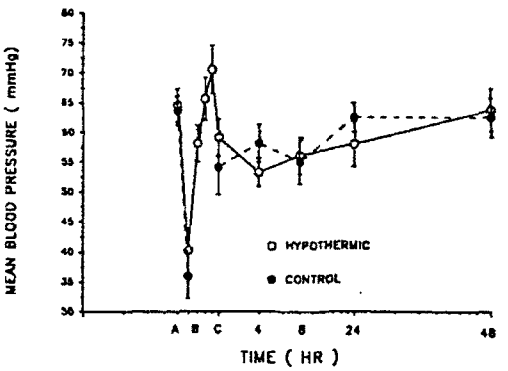
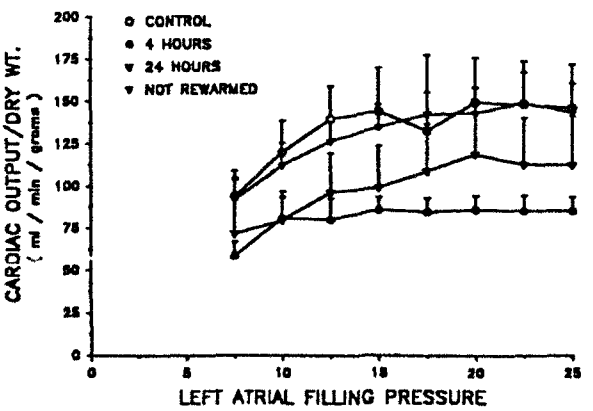


FIGURE 5



At various time intervals guinea pigs were anesthetized and their hearts were excised and hung on an isolated, perfused, working heart apparatus. Starling curves were generated. Figure 6 showed the changes in cardiac output/dry weight that

FIGURE 6



took place after hypothermia. The controls exhibit a normal Starling curve (open circles). Some animals were sacrificed when they reached the lowest body temperature (closed triangles). It would appear that the cooling does not immediately cause injury. However, a shift of the starling curve down to the right was seen (Figure 6). The differences between the 2 sets of curves were even clearer when the changes in left ventricular peak systolic pressure were compared (Figure 7).

FIGURE 7

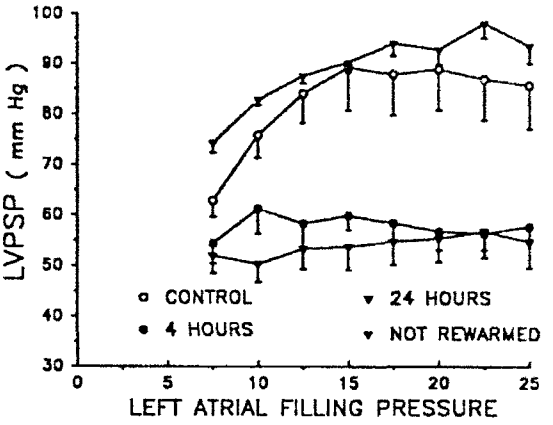


Figure 8 plots the changes in the dual product of cardiac output times peak systolic pressure as the left atrial filling pressure was increased (Figure 8). This was a measure of the energy utilization. It was obvious the some changes in the myocardium had taken place and they persisted for 24 hours. This was not observed in the O_2 consumption (Figure 9). DP/DT can be a measure of contractility, but it can also be influenced by

FIGURE 8

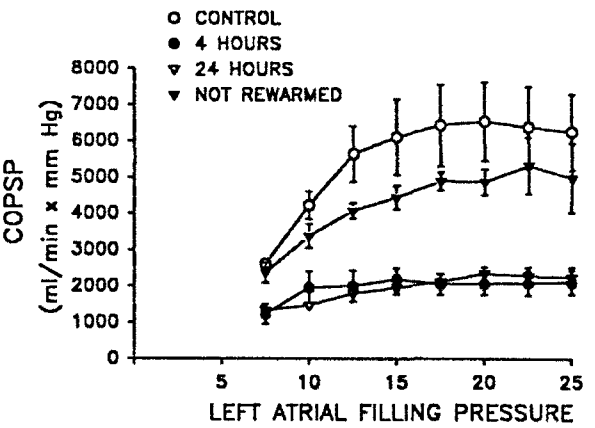


FIGURE 9

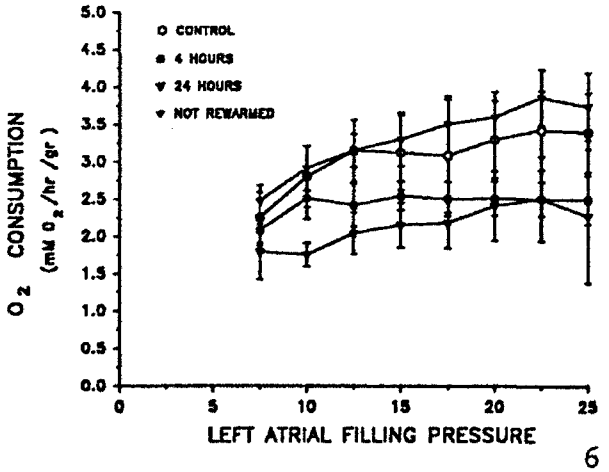
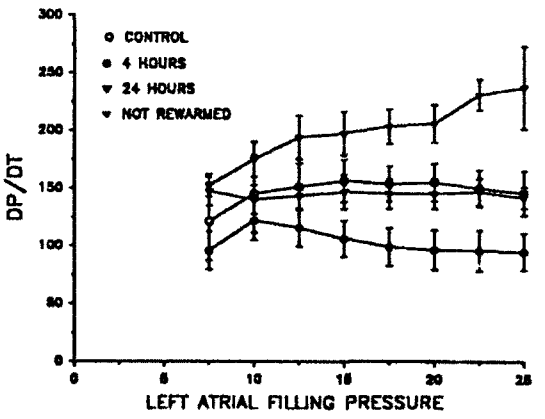


FIGURE 10



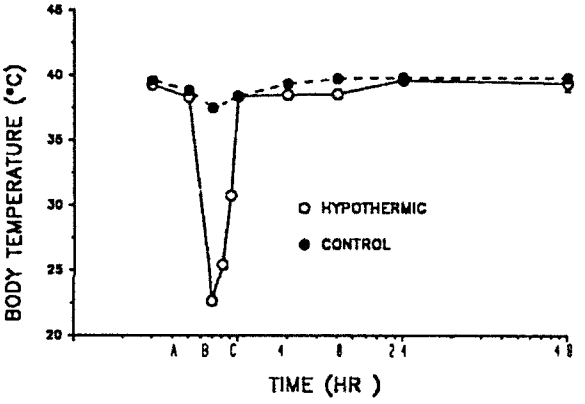
the compliance of the heart. It was interesting in Figure 10, that hearts taken from animals at lowest BT (not rewarmed) should exhibit the highest DP/DT while the lowest DP/DT were seen in hearts excised at 4 hours post rewarm.

Metabolic Studies

In the next group of animals free fatty acids (FFA), glucose and lactic acid were assayed during the precooling, rewarm and recovery period in addition to measuring heart rate, cardiac output, blood pressure, and body temperature at the same time.

Figure 11 shows the changes in core temperature for both control and hypothermic animals for this group with there being no difference from the first group observed. There were few changes in control temperature (closed circles). It was obvious that the guinea pigs in the ice-water bath had a rapid loss of temperature (point A). They were removed from the bath when the BT reached 25°C; however, their BT continued to fall. The lowest BT at which the guinea pig survived was 22.5°C (point B). After being removed from the bath, the animals were wrapped in a heating pad. After a delay of several seconds, the temperature rose rapidly and reached precooling temperature in about 45 minutes to an hour (point C). The guinea pigs were removed from the heating pad, placed in an individual cage and for 48 hours hemodynamic and metabolic measurements were made at various time intervals.

FIGURE 11



There was a small loss of body heat in both control and experimental animals even before the experimental animals were placed in the respective baths. This was due in part to the anesthesia. However, for the majority of the time, once they returned to the normal BT, it remained constant.

Figure 12 shows that anesthesia causes the same changes in mean arterial blood pressure (MABP) in the controls as the hypothermic animals. The lowest pressure was found right after

FIGURE 12

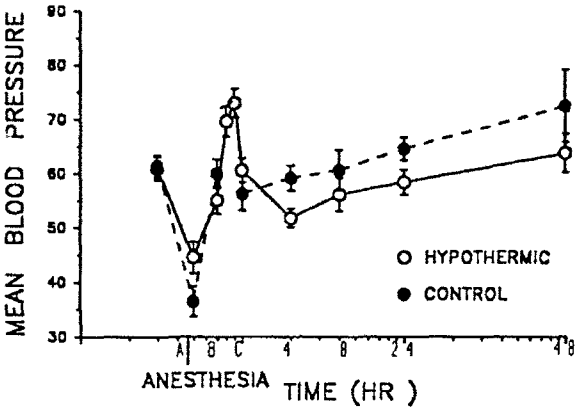
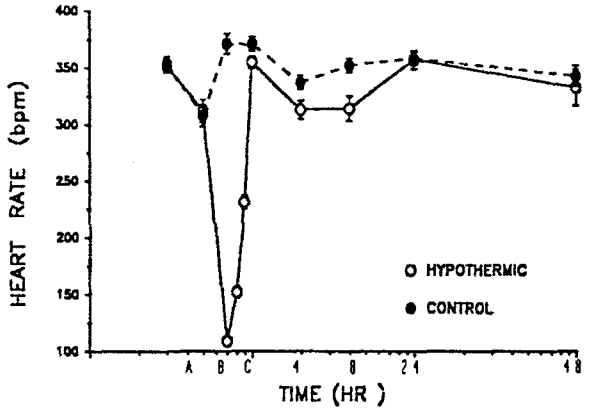


FIGURE 13



narcosis was induced (Figure 12). There appeared to be no effect of the hypothermia. However, while the experimental animals have very low pressure at the lowest temperature, it climbed back to normal as the temperature rose. The cardiac output, however, showed a difference between the hypothermic and the control. The difference persisted up to 8 hours and might be also at 24 hours (Figure 4). There were measurable differences in heart rate between control and the hypothermic animals. The heart rate dropped as the temperature dropped, probably due to a Q_{10} effect (Figure 13).

The FFA were depressed after rewarm (Figure 14). Glucose levels were elevated as expected (Figure 15), probably a reflection of a massive

FIGURE 14

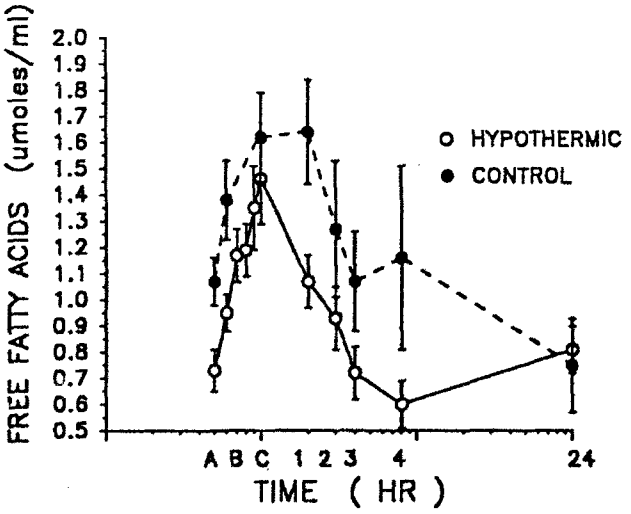
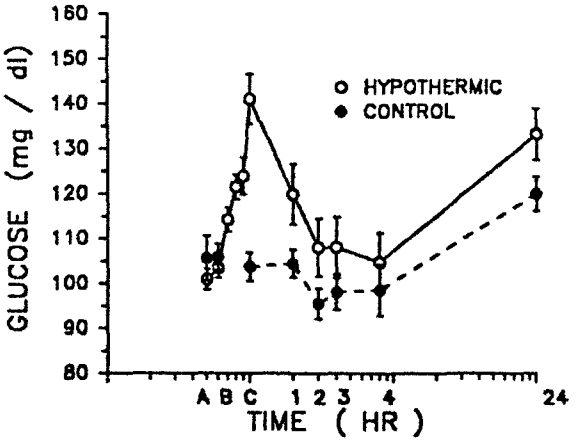


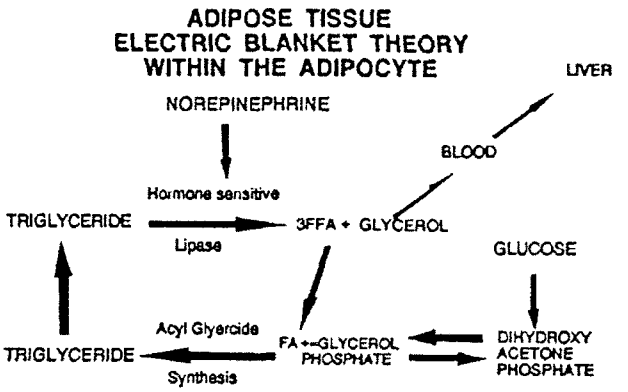
FIGURE 15



catecholamine release. The free fatty acid depression was probably due to fatty acid recycling (Futile Cycle) in the adipose tissue (Figure 16).

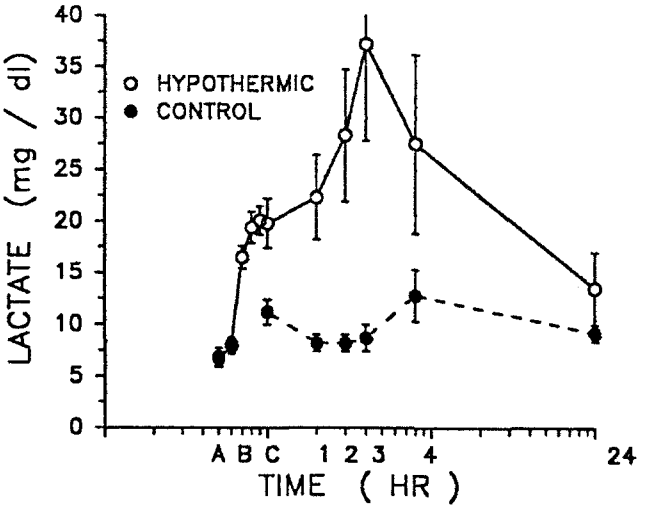
The elevated catecholamines caused a generalized peripheral vasoconstriction which decreases blood flow through skin and muscle. Blood lactates did not rise until peripheral flow was reestablished (Figure 17). Then the increased blood flow washed out the accumulated lactate. This was made evident in Figure 17 which showed an increase in lactate during rewarm. The lactate levels were so high they may be cardiotoxic. This might explain the myocardial depression of hypothermia.

FIGURE 16



FUTILE CYCLE- Produces heat much of which is preserved in the core.

FIGURE 17



CATECHOLAMINES RELEASE

The rapid changes in blood pressure and heart rate appeared to indicate a rapid rise in plasma catecholamines. The moderate changes in FFA and the more extensive changes in glucose may have been caused by the increase in epinephrine and perhaps a lesser increase in norepinephrine. Figure 18 indicated the exact opposite had taken place. The design of the experiment was the same as all the others. A control (CON) sample was taken. The animal was

anesthetized with Brevital®, and when it was fully unconscious, a precooled sample was taken (PRE). Point A sample was taken one minute after immersion in ice water and Point B was when the temperature reached 26°C. The animal was removed from the bath at 25°C and rolled in a heating pad. The temperature continued to drop to approximately 23°C. When the animal's temperature rose to 25°C another sample was taken. Additional samples were taken when the body temperature returned to the starting temperature (NBT), at 4 and 24 hours.

FIGURE 18

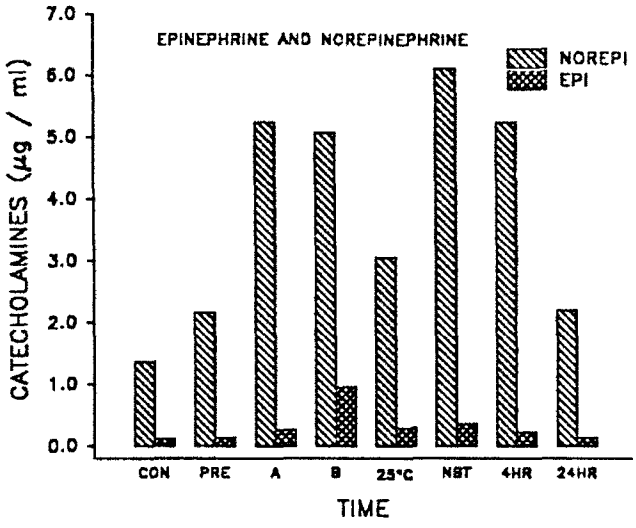


Figure 18 also shows a rapid increase in norepinephrine almost immediately upon immersion in the ice bath even though the guinea pig was narcotized. As the temperature fell, the heart rate slowed to very low rates. The catecholamines fell due to the Q_{10} effect in the adrenal medulla. There was little circulation perfusing the adrenal modula and little chemical production going on. However, when the body temperature rose to NBT, the plasma norepinephrine concentration rose rapidly and was four-fold higher than control. In the guinea pig, epinephrine levels were usually very low and therefore epinephrine changes were more modest. They were maximally elevated at Point B and were still slightly elevated when the animal returns to NBT. They were back to control concentrations at 24 hours.

One would expect a rise in heart rate and a decrease in cardiac output with the small rises in epinephrine and the large increases in norepinephrine. However, these changes were not observed (Figures 3,4). By 24 hours both catecholamine levels had returned to control level.

Since norepinephrine is a powerful stimulus of hormone sensitive lipase, the five-fold increase in plasma norepinephrine should have elevated the FFA to very high levels. What we actually saw was a modest increase in both groups until 3 hours post NBT

(Figure 14). Four hours after return to NBT the FFA returned to initial levels even though the norepinephrine concentration remained elevated. We do not know if this tolerance effect was due to receptor saturation or low cofactor concentration. The increase in epinephrine seen at Points A, B and 25°C, which occured as the body temperature rose, did increase the plasma glucose. The reason for this was epinephrine increased cyclic AMP which stimulated glycogen breakdown and increased hepatic glucose output. This in turn may have stimulated insulin release. The insulin promoted more glucose movement into the adipose tissue which increased triglyceride synthesis and decreased FFA release.

EFFECT OF VARIOUS RESUSCITATION FLUIDS ON HYPOTHERMIC SHOCK

Shock, when it is the result of acute and severe accidental hypothermia, can produce subtle injuries to several organs. The manifestations of these injuries were difficult to observe because of compensatory mechanisms of the heart. These symptoms persisted many hours past the return to normal body temperature. The severity of these organ dysfunctions was not always great but coupled with other changes could have jeopardized the survival of the victim. One of the purposes of these investigations was to uncover the mechanisms and determine proper therapeutic procedures to stop and reverse this pathological process. One of the therapeutic interventions studied was fluid resuscitation, which was applied during the short rewarm period. We had shown that following hypothermia and rewarm a cardiac dysfunction persisted over 48 hours. This dysfunction was hidden in the whole intact animal because of the cardiovascular compensatory mechanism. However, if the heart was isolated and perfused, hearts from hypothermic animals exhibited flat Starling curves. In other words, hearts from these hypothermically shocked animals lose their physiologic reserve. The question was, "Does the composition and volume of the resuscitation fluid influence the development of the cardiac dysfunction?"

The usual guinea pig model with indwelling carotid artery and jugular vein catheters were anesthetized with Brevital®, a fast-acting barbiturate. When fully narcotized and unconscious, the

animals were immersed in an ice-distilled water bath. Core temperature changes were monitored from a second carotid catheter, containing a microthermistor which had been maneuvered into the thoracic aorta. Observations were made and samples collected. When the body temperature reached its lowest temperature (22°C-23.5°), the aniamls were rolled in a heating pad and resuscitated with various fluids. When the body temperature returned to 38.5°C (NBT) they were removed from the heating pads and fluid resuscitation was stopped. Cardiac output, using thermal dilution technique, heart rate and blood pressure were recorded at each time point. Blood FFA, glucose and lactic acid were determined from samples taken at the same time periods. The infusate was <u>not</u> heated. The infusion rate was 0.25 ml/min. There were four groups: non-resuscitated (NR), normal saline (.85%) (NS), Ringers lactate (RL) and Ringers acetate (RA) solution.

FIGURE 19

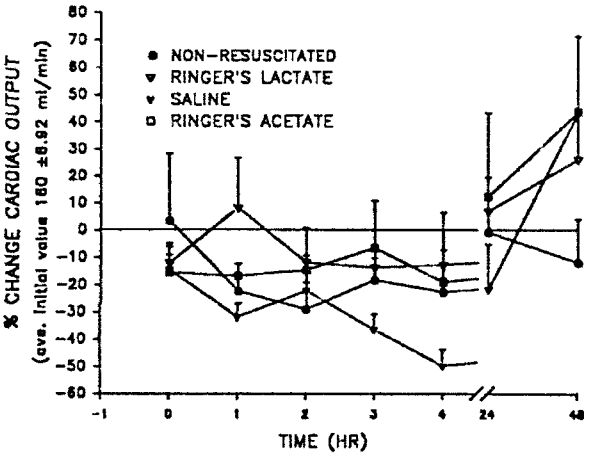
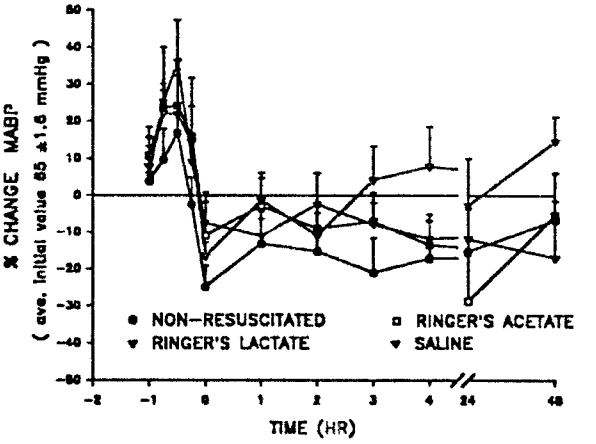


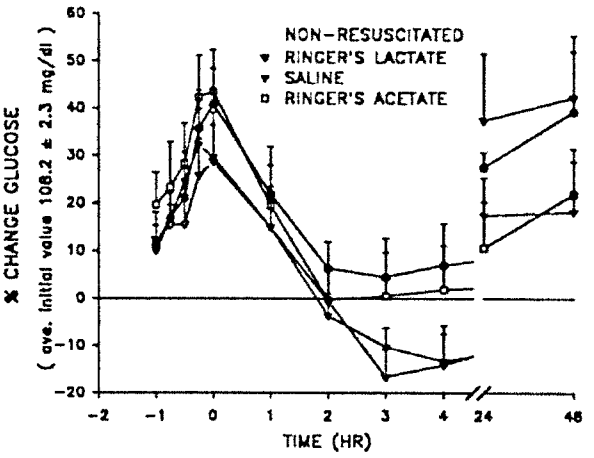
Figure 19 shows the percent change of the cardiac output when normalized to the pre-anesthetized precooled value. All groups expect NR had about 15% decrease of the cardiac output at NBT (O time), while NR which had little or no change. At 1 hour post NBT, the Ringers acetate group (RA) and the non resuscitated (NR) group dropped to about 25% while the Ringers lactate group (RL) appeared elevated. The saline group fell to 33% of the precooled level. At 3 and 4 hours the saline group continued to fall while the other groups had a slight increase. Twenty-four hours post cooling, the cardiac output of all groups except saline returned to precooled values.

FIGURE 20



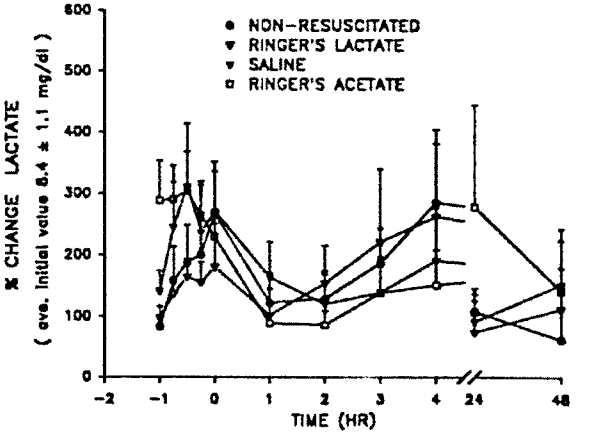
After a small initial rise during early cooling, the mean arterial blood pressure (MABP) fell and remained depressed through NBT (0 time) in all groups (Figure 20). At 1,2 and 3 hours post NBT all resuscitated groups were no different from each other or from the precooled values. The non resuscitated (NR) remained depressed four hours post recovery. While there was no clear difference between the resuscitated groups at 24 and 48 hours post recovery, the NR remained depressed even at 24 hours.

FIGURE 21



In most types of shock, blood glucose levels were elevated early because of catecholamine release. Hypothermic shock was no different (Figure 21), peaking at O time. They fell to precooled control values by 2 hours. The NR and RA remained at control levels at 4 hours while NS and RL were depressed. Blood glucose concentration in all groups were elevated 24 and 48 hours post cooling.

FIGURE 22

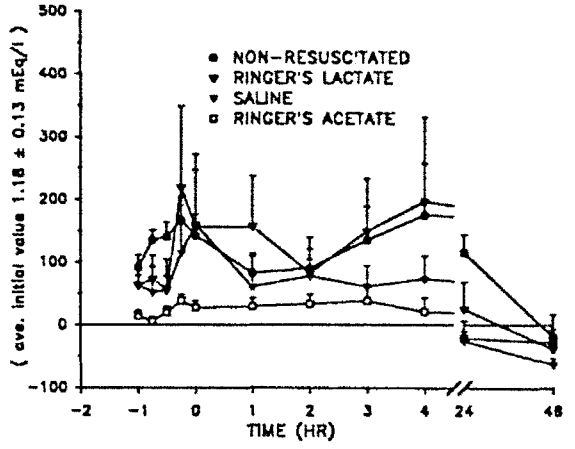


Since blood lactate levels were very labile, it was not surprising that this concentration was elevated in all groups, but its elevation was variable (Figure 22). At 1 hour post recovery, the blood lactic acid concentration fell in all groups. Remember, fluid resuscitation was stopped at "O" time when the body temperature returned to normal (NBT). At 4 hours NS and RA did not change from the previous samples while RL and NR appeared to be elevated. At 24 and 48 hours while slightly elevated, there were no differences of lactate concentration between the groups. Because the initial lactate levels were low,

8.1 mg/dl, small changes were magnified. A 50% change in lactate was only an increase to 12 mg/dl.

All groups exhibited general increase in plasma free fatty acid (FFA) except RA (Figure 23). RA showed a slight elevation during 4 hours post recovery and no change from controls at 24 and 48 hours. At 4 hours RL and NR were elevated over 150%. NS was increased somewhat less. At 24 hours there were no differences among the resuscitated groups while NR remained elevated. Forty-eight hours post recovery all lactate levels were at or less than control levels.

FIGURE 23



It was our thesis that even a brief exposure of myocytes to high levels of lactate during shock periods brings about the cardiac dysfunction we observed. We had not clearly observed the cardiac dysfunction in the whole animal following hypothermic shock because of the hemodynamic compensatory mechanisms. We could not relate changes in cardiac output to the nature of the resuscitation fluid nor did the MABP show any difference. The metabolic changes were clearer. The lactate levels were higher and remained elevated in RL and NR at 4 hours. This may be when the damage was done. The elevated FFA may have reflected the stress of the animals at the time of the highest lactate levels. Both lactate and FFA may in part have contributed to the cardiac dysfunction. The lactate and FFA in RA and NS seem to be lower. This may account for their increased survival.

IN VITRO EXPERIMENTS

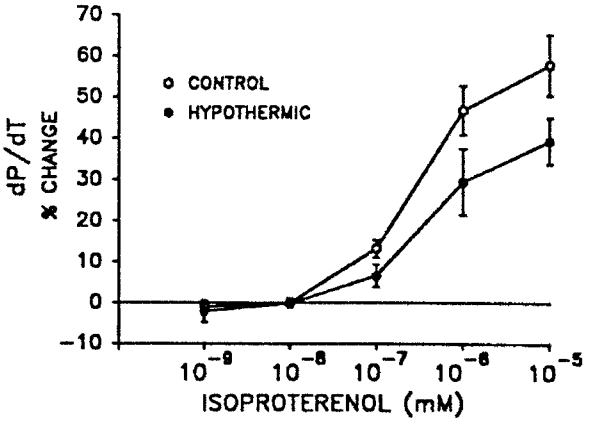
When hearts from hypothermic animals were isolated and perfused, they exhibit depressed Starling curves (Figure 6). In other words, hearts from these hypothermically shocked animals had a decreased physiologic reserve. Catecholamines released in response to the stress of hypothermia contributed to this reserve when the contractility of the heart was decreased. They helped

maintain a sufficiently high blood pressure to perfuse the critical organs when the heart began to fail. However, even with catecholamine support, the heart failed when its temperature fell below some critical point and its own chemical production of energy was too low. This may have resulted from a Q_{10} effect. It appeared that after the body temperature had returned to normal, the catecholamines remained elevated for 4 to 24 hours without the usual adrenergic manifestations of: (1) elevated heart rate, (2) peripheral vasoconstriction and (3) elevated blood FFA and glucose levels. It appeared that the catecholamine sensitive organs had decreased sensitivity. This could be, in part, one mechanism of this cardiovascular failure.

SENSITIVITY OF CATECHOLAMINES

Guinea pigs prepared with indwelling catheters and thermistors were temporarily anesthetized with a short acting barbiturate, Brevital®, and immersed neck-deep in ice-water until their core temperature fell to 25.5°C and rewarmed in the usual way. In these experiments, some of the guinea pigs were anesthetized at 4 hours following return to normal core temperature. The hearts of these animals were excised and placed on a perfused working heart apparatus. We then tested the function of these hearts with a variety of preloads. After all measurements were made, isoproterenol was infused into the left atrium and dose response curves were constructed.

FIGURE 24



In order to test the sensitivity of the adrenergic receptors of the myocardium, hearts of control and hypothermic guinea pigs were isolated and were infused at different dosages of isoproterenol. Control hearts showed no response at dosages up to 10^{-8} M. At 10^{-7} M, max dp/dt rose 10% and at 10^{-6} M it increased 40%. At 10^{-5} M, it had similar response as the concentration 10^{-6} M, with an increase in isolated spontaneous arrhythmias. Dosages higher than 10^{-5} M produced

ventricular fibrillation (Figure 24). When 10^{-7} M was applied to the experimental hearts, they fibrillated. The same pattern was

FIGURE 25

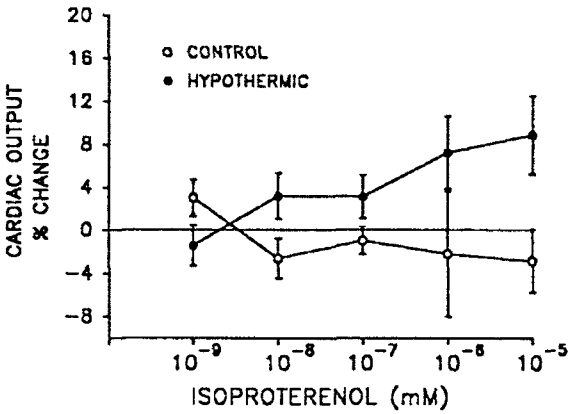
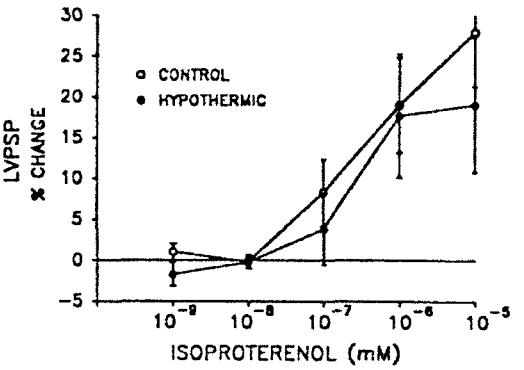


FIGURE 26



seen for cardiac output (Figure 25) and left ventricular peak systolic pressure [LVPSP] (Figure 26).

It appeared that hearts of the experimental guinea pigs are less sensitive to β-adrenergic stimulation; instead of stimulating increases in heart rate, blood pressure and contractility, it decreased its ability to respond to isopreterenol. The mechanism of this phenomenon was uncertain. Also, a much smaller dose (10^{-7} M) produced fibrillation. It was interesting that this dose was the lowest effective dose in the controls. It would appear that the β-adrenergic receptors had down-regulated due to saturation of the most sensitive receptors. Also, synthesis of receptors may have been slowed because of the very low temperature. Some of the β-adrenergic effects of catecholamines have been muted by the combination of both of these events. Thus, other adrenergic effects, usually obscured by β effectors may surface. However some of the less apparent effects, for example vasoconstriction of the coronary circulation, can surface and may be partially responsible for the fibrillation seen in the hearts of the experimental animals.

CATECHOLAMINES AT VARIOUS TIME INTERVALS POST HYPOTHERMIA

Guinea pigs were prepared in the usual way, with a catheter in a jugular vein and another in a carotid artery. A microthermistor was placed in the arch of the aorta as previously described. On the day of the experiment, they were temporarily anesthetized with

a short acting barbiturate, Brevital®, and immersed neck-deep in ice water until their core temperature fell to 25.5°C. This process took 6-7 minutes. Observations were recorded and blood samples taken at 30 seconds and then each minute. The animal was then wrapped in a heating pad and warmed until the core temperature returned to 38.5°C. Even though the guinea pig was being warmed, during the first few minutes the temperature continued to fall. When it reached its lowest point (LBT), blood samples for catecholamines were drawn and recordings of heart rate, blood pressure and BT were obtained. Then BT began to rise slowly. When it reached 38.5°C, approximately 45 minutes later blood samples were again drawn and recordings were again obtained. This point was considered 0 time. Recordings and blood samples were also taken at 1, 2, 3, 4 and 24 hours from 0 time.

Figure 27 shows the changes in BT from control samples, through the cool down, rewarm and 1, 2, 3, 4 and 24 hours after return to normal temperature, as well as a maximized portion of the interval between the control and LBT. At 30 seconds the core BT did not fall. Even at 1 minute post immersion there was only a small drop. At 2 minutes, there was a significant drop which continued to the LBT. The control animal, immersed in warm (38-39°C) water had a BT

FIGURE 27

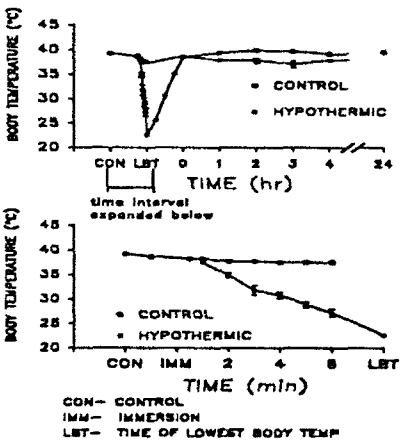


FIGURE 28

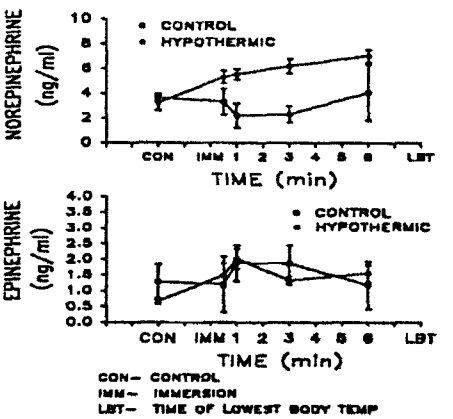


FIGURE 29

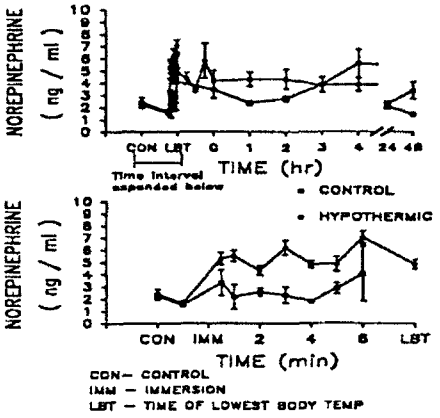
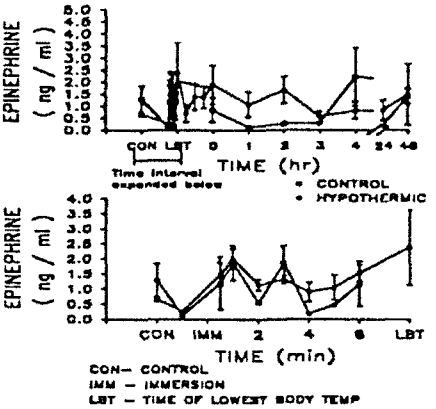


FIGURE 30



decrease of only a few degrees which was restored with a few minutes exposure to the heating pad.

During cool-down, plasma norepinephrine concentration (NorEpi) steadily rose (Figure 28). At 6 minutes post immersion, when the body temperature reached 25.5°C, Norepi levels rose to 2 times that of control. Norepinephrine levels remain elevated two-fold until 4 hours post rewarm (Figure 29). Epinephrine concentrations varied widely, therefore no conclusion could be drawn (Figure 30).One would expect the FFA to be elevated with the high norepinephrine levels. However,this was not observed (Figure 31). While the blood glucose levels of both the hypothermic and control animals rose during cool down, they returned to normal values at 0 time in the controls and fell to low levels at 2 hours post return reflecting insulin release (Figure 32). By 4 hours they are essentially normal. The hypothermic animals peak at 0 time, returning to normal by 4 hours. Lactate rises steadily in the hypothermic animals until LBT. There is a slight elevation of

FIGURE 31

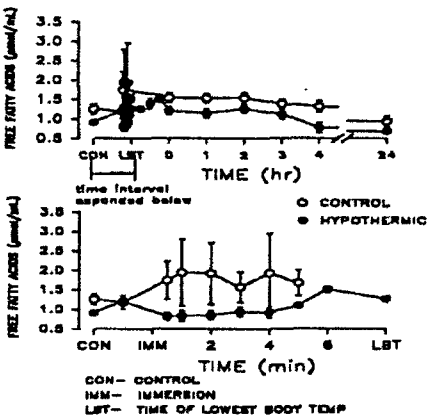


FIGURE 32

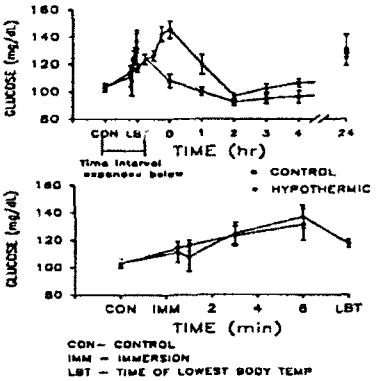
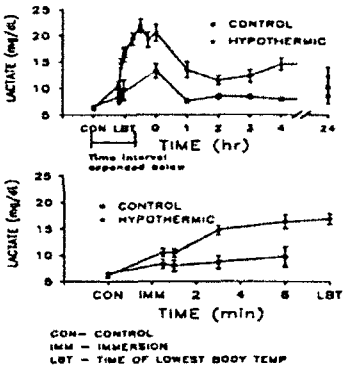


FIGURE 33

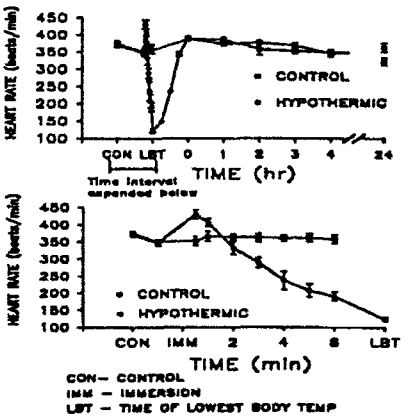


control lactate levels, until time 0 presumably due to the anesthetic agent. While the hypothermic increases are large, they reflect ischemia in the peripheral tissues which wash out on rewarming (Figure 33).

SUMMARY

The results of the metabolic studies suggest that while there are very large increases in circulating catecholamines, the catecholamines were less sensitive in the hypothermic animals after rewarm. Heart rate was elevated almost immediately upon immersion in cold water and did not fall until 2 minutes after immersion. When the BT returned to normal (0 time) so did the heart rate (Figure 34), even though the catecholamine levels were very high. It would appear that the adrenergic receptors are down-regulated and seemed less sensitive.

FIGURE 34



This may be the reason for lack of elevation of the FFA. However, there may be another explanation. The "Adipose Tissue Electric Blanket Theory" suggests two roles for triglycerides in the subcutaneous adipose tissue (Figure 16): 1) It is a very good insulator and preserves the heat in the core; 2) The cycling of triglyceride, the major component in adipose tissue, is hydrolyzed to 3 FFA and glycerol and then reesterified into

triglyceride. The product of this cycle is heat. Hormone sensitive lipase controls the lipolysis of the triglycerides. Norepinephrine controls hormone sensitive lipase activity. The reason for the lack of elevation of plasma FFA might be an increase in the reesterification of fatty acids. However, the hypothermic animals' adrenergic receptors appear to be downregulated and these animals have a difficult time maintaining core temperature even 4 hours after rewarm. It is likely that the hormone sensitive lipase is depressed, the futile cycle is slowed, and heat production within the adipose tissue is depressed in these hypothermic animals.

In order to study these phenomena we would like to measure both plasma FFA and glycerol to determine the retention of FFA by adipose tissue. Since the freed glycerol cannot be rephosphoralated within the adipose tissue, glycolysis must supply the α glycerophosphate, so that the ratio of glycerol to FFA in the plasma is a reflection of adipose tissue reesterification. In this way we would be able to tell whether the loss of temperature regulatory ability is due to a short circuit of the electric blanket.

The changes we observed in the metabolic samples (FFA, glucose and lactate) are related to the elevation of NorEpi. When the energy bearing metabolites cannot be mobilized by catecholamines due to down-regulation and, the futile cycle of triglycerides within the adipose tissue does not produce the heat required, then the organism can no longer thermoregulate appropriately.